ClinGen *TP53* Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1.2

This version specified for the following genes: *TP53*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50013

Release Notes/Changes from v1: 1) Corrected formatting and typos 2) Provided clarifying language to facilitate understanding and application of rule specifications (April 2021)

Gene	Disease (MONDO ID)	Clinically significant transcript
TP53	**Li-Fraumeni syndrome (0007903)**	**NM_000546.4**

Pathogenic Criteria		
Criteria	**Original Criteria Description**	**Specification(s)**
Very Strong Criteria		
PVS1	Null variant in a gene where LOF is a known mechanism of disease	Defer to SVI recommendations
PS2_PM6_Very Strong	*De novo*, proven or assumed	Use SVI point system table. *See Cancer Criteria List & *TP53* Point Table at end of document. ≥4 points (ex. – 2 cancers in two probands from the strong criteria list or 4 cancers from 4 probands from the moderate criteria). For probands with multiple cancers, use the most specific/highest weight cancer to determine point for that proband.
Strong Criteria		
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	Must confirm there is no difference in splicing using RNA data. Can only compare to variants asserted as pathogenic by the ClinGen *TP53* EP.
PS2_PM6_Strong	*De novo*, proven or assumed	Use SVI point system table. *See Cancer Criteria List & *TP53* Point Table at end of document.

		2-3 points (ex. – 1 cancer from the strong criteria list or 2 from the moderate criteria list)
PS3	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a low functioning allele (<= 20% activity) **AND:** • Evidence of dominant negative effect (DNE) + evidence of LOF from Giacomelli, et al data **-OR-** There is a 2nd assay showing low function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays)
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Use proband counting system described below in text. PS4 = 4+ points
PP1_Strong	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Cosegregation must be observed ≥7 meioses in >1 family in to apply this rule.
Moderate Criteria		
PS1_Moderate	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	Must confirm there is no difference in splicing using *in silico* modeling data using a splice metapredictor (SpliceAI, VarSEAK, etc). Can only compare to

		variants asserted as pathogenic by the ClinGen *TP53* EP.
PS2_PM6_Moderate	*De novo,* proven or assumed	Use SVI point system table. *See Cancer Criteria List & *TP53* Point Table at end of document. 1 point (for 1 cancer from the moderate criteria list)
PS3_Moderate	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	A) Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a partially functioning allele (>20- and <=75% activity) **AND:** ● Evidence of DNE + evidence of LOF from Giacomelli, et al data. **-OR-** ● There is a 2nd assay showing low function *Do not use code with conflicting evidence.*
		B) No transactivation assays (IARC classification based on data Kato et al, 2003) available **BUT:** ● Evidence of DNE + evidence of LOF from Giacomelli, et al data. **-AND-** ● There is a 2nd assay showing low function *Do not use code with conflicting evidence.*
PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Use proband counting point system described in text below. PS4_moderate = 2-3 points

PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation	This rule can be applied to variants in hot spots (codons 175, 245, 248, 249, 273, 282), but not to variants within functional domains. Use transcript NM_000546.4. Also use rule for variants with ≥10 somatic observations cancerhotspots.org (v2)
PM3	For recessive disorders, detected in trans with a pathogenic variant	Does not apply
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	This rule should not be used at this time due to limited data.
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Multiple pathogenic variants (≥2) at that residue using the requirements specified below (excluding known hot spots) would be required. Grantham should be used to compare the variants. At least one of the new variants must be equal or worse than known pathogenic variant. Splicing should be ruled out. Can only compare to variants asserted as pathogenic by the ClinGen *TP53* EP. Rule cannot be used in conjunction with PM1.
PP1_Moderate	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Cosegregation must be observed in 5-6 meioses in 1 family to apply this rule.
PP3_Moderate	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	PolyPhen2 and SIFT *in silico* modeling programs should not be used for this gene.

		Missense variants: aGVGD (Zebrafish; Class C65 required) and BayesDel (score ≥ 0.16)
Supporting Criteria		
PS4_Supporting	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Use proband counting point system described in text below. PS4_Suppporting = 1 point
PM2_Supporting	Absent in population databases	Variant needs to be absent from controls.
PM5_Supporting	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Grantham should be used to compare variants. The new variant must be equal or worse than known mutation. Splicing should be ruled out. Rule cannot be used in conjunction with PM1.
PS2_PM6_Supporting	De novo, proven or assumed	Use SVI point system table. *See Cancer Criteria List & *TP53* Point Table at end of document. 0.5 point (1 cancer from the moderate criteria list)
PP1	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Cosegregation must be observed in 3-4 meioses in 1 family to apply this rule.
PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease	This rule should not be used due to the high frequency of benign missense variants.

PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	PolyPhen2 and SIFT *in silico* modeling programs should not be used for this gene. Concordance of two predictors is recommended for this gene: ● Missense variants: aGVGD (Zebrafish; Class C25 and higher are considered evidence of pathogenicity) and BayesDel (scores $\geq$ 0.16 are considered evidence of pathogenic) ● Splicing variants: Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc).
PP4	Patient's phenotype and/or family history is highly specific for a disease with a single genetic etiology	Use modified PS4 criteria instead of PP4 code
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.	Do not use

Benign Criteria		
Criteria	**Original Criteria Description**	**Specification(s)**
Stand Alone Criteria		
BA1	Allele frequency is greater than expected for disorder	Frequency cutoff of 0.1% minimum of 5 alleles present in the population
Strong Criteria		
BS1	Allele frequency is greater than expected for the disorder	Frequency cutoff of 0.03%; minimum of 5 alleles present in the population
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	Observed in ≥8 cancer free 60+ year old females obtained from the same data source
BS3	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that show retained function (76-140% activity) or supertransactivation function **AND:** • No evidence of DNE + no evidence of LOF from Giacomelli, et al data. **-OR-** There is a 2nd assay, including colony formation assays, apoptosis assays, tetramer assays, growth suppression and knock-in mouse models demonstrating retained function.
BS4	Lack of segregation in affected members of a family	Variant segregates to opposite side of the family who meets LFS criteria **- OR -**

		Variant is present in ≥3 living unaffected individuals (at least 2 of which should be female) above 55 years of age.
Supporting Criteria		
BS2_Supporting	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	Observed in 2-7 cancer free 60+ year old females obtained from the same data source
BS3_Supporting	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a partially functioning allele (>20% and <=75% activity) **AND:** ● No evidence of DNE + no evidence of LOF from Giacomelli, et al data. **-OR-** ● There is a 2nd assay demonstrating retained function *Do not use code with conflicting evidence.*
		No transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) available **BUT:** ● No evidence of DNE + no evidence of LOF from Giacomelli, et al data. **-AND-**

		● There is a 2nd assay showing retained function
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	Do not use
BP2	Observed *in trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed *in cis* with a pathogenic variant in any inheritance pattern	● Variant is observed *in trans* with a pathogenic or likely pathogenic *TP53* variant (phase confirmed) OR 3 or more observations when phase is unknown with at least two different pathogenic/likely pathogenic *TP53* variants
BP3	In-frame deletions/insertions in a repetitive region without a known function	Do not use
BP4	Multiple lines of computational evidence suggest no impact on gene/gene product	Missense: aGVGD (zebrafish; Class C0 or C15 is considered evidence of non-pathogenicity) and BayesDel <0.16 is considered evidence on non-pathogenicity Splicing: Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc).
BP5	Variant found in a case with an alternate molecular basis for disease	Do not use
BP6	Reputable source recently reports variant as benign, but the evidence is not available to	Do not use

	the laboratory to perform an independent evaluation	
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc).; If a new alternate site is predicted, compare strength to native site in interpretation.

RULES FOR COMBINING PATHOGENIC CRITERIA**

PATHOGENIC

1. 1 Very Strong AND
    a. ≥1 Strong OR
    b. ≥2 Moderate OR
    c. 1 Moderate and 1 Supporting OR
    d. ≥2 Supporting
2. ≥2 Strong OR
3. 1 Strong AND
    a. ≥3 Moderate OR
    b. 2 Moderate AND ≥2 Supporting OR
    c. 1 Moderate AND ≥4 Supporting

LIKELY PATHOGENIC

1. 1 Very Strong AND
    a. 1 Moderate OR
    b. 1 Supporting
2. 1 Strong AND 1-2 Moderate OR
3. 1 Strong AND ≥2 Supporting OR
4. ≥3 Moderate OR
5. 2 Moderate AND ≥2 Supporting OR
6. 1 Moderate AND ≥4 Supporting

RULES FOR COMBINING BENIGN CRITERIA**

Benign*

1. 1 Stand-Alone OR
2. ≥2 Strong

Likely Benign*

1. 1 Strong AND
   a. 1 Supporting OR
   b. No pathogenic evidence codes
2. ≥2 Supporting

*PM2_Supporting should not be used to pull variant to VUS classification if there are sufficient evidence codes for variant to meet Benign or Likely Benign.
**A single supporting evidence code in either direction should not be considered conflicting evidence for curation of the variant otherwise meets criteria for a P/LP or B/LB variant. Use caution and follow written guidance for rule combination when variants meet criterion for the following combinations: BA1/BS1 and PS4; PM1 and PM5.

This version specified for the following genes: *TP53*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50013

ACMG Classification Rule Specifications for *TP53*

SUMMARY OF CLASSIFICATION CRITERIA

(TP53 specified rules are in red. Rules in grey are not applicable to TP53.)

PATHOGENIC

Very Strong

PVS1 – Null variant in gene with established LOF as disease mechanism

Strong

PS1 - Same amino acid change from different DNA change

PS2 – *De novo* with paternity and maternity confirmed

PS3 – Well-established in vitro or in vivo, functional studies supportive of a damaging effect

PS4 - Prevalence of variant in affected individuals is significantly increased over controls.

Moderate

PM1 – Present in functional region without benign variation

PM2 – Rarity/Absence in control populations

PM3 - Detected *in trans* with a pathogenic variant

PM4 – Protein length changes

PM5 – Missense change at same codon as another pathogenic missense variant

PM6 – Assumed *de novo* with specifications as recommended by SVI WG

Supporting

PP1 – Cosegregation with disease in multiple affected family members

PP2 - Missense variant in a gene with low rate of benign missense variation

PP3 –Multiple lines of computational evidence supporting a deleterious effect

PP4 – Phenotype and family history specific for disease with single genetic etiology

PP5 - Reputable source reports as pathogenic

BENIGN

Stand-Alone

BA1 – Allele frequency cutoff ≥0.1% (99.99% CI w/subpopulation w/min of 5 alleles)

Strong

BS1 – Allele frequency cutoff ≥0.03% (99.99% CI w/subpopulation w/min of 5 alleles)

BS2 - Observed in an increased frequency of cancer free females

BS3 – Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing

BS4 - Non-segregation in affected relatives

Supporting

BP1 - Missense variant in gene where only LOF causes disease

BP2 – Bi-allelic variants for a fully penetrant disorder

BP3 - In-frame deletions/insertions in a repetitive region without a known function

BP4 - Multiple lines of computational evidence support no impact

Related publication(s): PMID: 33300245 **Date Approved:** August 6, 2019

BP5 – Variant found in a case with an alternate molecular basis for disease
BP6 - Reputable source reports as benign
BP7 - Synonymous variant for which splicing prediction algorithms predict no impact

VERY STRONG EVIDENCE OF PATHOGENICITY

PSV1 Null variant in a gene where LOF is a known mechanism of disease. Null variants to include truncating variants, canonical splice sites, exon gross deletions, intragenic exon tandem duplications, and the initiation codon

- Use the PVS1 workflow guidance provided in Tayoun et al. 2018 (PMID: 30192042)

STRONG EVIDENCE OF PATHOGENICITY

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

- PS1:

  - This rule code can only be used to compare to variants asserted as pathogenic by the ClinGen *TP53* EP.
  - Must confirm there is no difference in splicing using RNA data.

- PS1_Moderate:

  - This rule code can be used if there is no difference in splicing using *in silico* modeling data using a splice metapredictor (SpliceAI, VarSEAK, etc).
  - This rule code can only be used to compare to variants asserted as pathogenic by the ClinGen *TP53* EP.

PS2/PM6 *De novo* variant in a patient with the disease and no family history.

- Apply this code when a *de novo* variant is identified. The *TP53* point system is described below and is based on whether maternity and paternity have been confirmed and the type of cancer(s) seen in the proband. For probands with multiple cancers, use the most specific/highest weight cancer to determine point for that proband. Use the SVI scoring system see below) to determine the strength of the PS2 code.

(See box of associated criteria and points system on next page)

Strong associated LFS criteria • 2 points for probands with one of the following cancers when maternity and paternity are confirmed • 1 point for probands with one of the following cancers when parental testing is not available	• Breast cancer (IDC & DCIS) <31 years of age • Choroid plexus carcinoma • Adrenocortical adenoma or carcinoma <18 years of age • Rhabdomyosarcoma <46 years of age • Osteosarcoma <46 years of age
Moderate associated LFS criteria • 1 point for probands with one of the following cancers when maternity and paternity are confirmed • 0.5 point for probands with one of the following cancers when parental testing is not available	• Breast cancer >30 and <50 years of age • Malignant brain tumors (excluding optic gliomas) <46 years of age • Primary lung cancer <46 years of age • Adrenocortical adenoma or carcinoma ≥18 and <50 years of age • Rhabdomyosarcoma and osteosarcoma >45 years of age • Other sarcomas (e.g. malignant phyllodes tumor, leiomyosarcoma, liposarcoma) <60 years of age ○ Exclude dermatofibrosarcoma & Ewing sarcoma • Hypodiploid ALL (Specifically low-hypodiploid 32-39 chromosomes)

Supporting PM6_Supporting	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4

PS3 Well-established in vitro or in vivo, functional studies supportive of a damaging effect on the gene or gene product

- Kato et al, 2003 (PMID: 12826609) data performed the best on our test set of variants. These data thus remain the main functional assay underlying the classification.
- Giacomelli et al, 2018 (PMID: 30224644) assays are systematic and are available for more than 8000 mutants. When using cut-offs derived from original publication data (optimal cut-offs separating silent and common cancer variants), they show good concordance with other ssays. The combination of two assays (DNE + LOF) show better performance than LOF alone (0.88 vs 0.73) and DNE alone (0.88 vs 0.70) when tested on the test set of variants. The performance of DNE+LOF was close to the one of Kato data (0.88 vs 0.94). There are >5900 variants included in Giacomelli dataset (including 378 silent and 414 stopgain variants) that have no Kato data. Over 400 of these variants have at least one entry in the IARC database (somatic). Giacomelli DNE+LOF class can thus be used to support and complement Kato data. Giacomelli DNE+LOF data could be used when there is no Kato data but with a moderate code because of the less robust cut-offs of the assays.

- Non-systematic assays are harder to interpret but if there are several of them and if all suggest benign or pathogenic, they should be taken into account. A large proportion of these assays is documented in the IARC database and thus be easily found by curators.
- Kotler data are available for a large number of mutants, but only for mutants within the DNA binding domain. They will be used as other non-systematic LOF assay.
- For variants that are partially functional by Kato, Giacomelli LOF/DNE can be used as results correlate well with Kotler data and quantitative model outputs.

Data supporting Functional classes:

IARC Transactivation class: IARC classification based on transactivation assays in yeast from Kato et al., (2003): non-functional is <=20% activity; partially functional is >20% and <=75% activity; functional and supertrans are >75% activity.

Giacomelli et al. (2018): IARC classification based on growth suppression assays in A549 human cells; DNE+LOF is p53WTNutlin3 Z-score >= 0.61 and Etoposide Z-score <= -0.21; noDNE+noLOF is p53WTNutlin3 Z-score < 0.61 and Etoposide Z-score > -0.21.

Other assays (available in IARC database or original publications): in vitro growth assays in H1299 human cells from Kotler et al., (2018) with RFS score >= -1.0 for LOF and RFS score < -1 for noLOF; or colony formation assays, growth suppression assays, apoptosis assays, tetramer assays, knock-in mouse models.

- PS3: Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a low functioning allele (<20% activity) **AND:**
  - There is evidence of dominant negative effect (DNE) + evidence of LOF from Giacomelli, et al data

-OR-

- There is a 2nd assay showing low function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays)

- PS3_Moderate: Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a partially functioning allele (>20-and <=75% activity) **AND:**

  - There is evidence of DNE + evidence of LOF from Giacomelli, et al data.

-OR-

  - There is a 2nd assay showing low function

Do not use code with conflicting evidence

- PS3_Moderate: When there are no transactivation assays (IARC classification based on data Kato et al, 2003) available **BUT:**

  - There is evidence of DNE + evidence of LOF from Giacomelli, et al data.

-AND-

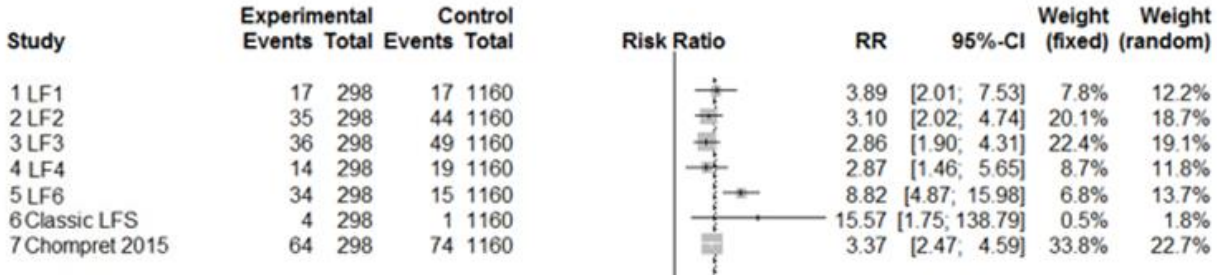
  - There is a 2nd assay showing low function

Do not use code with conflicting evidence

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.

- There are two widely used criteria used for assessing the likelihood of Li-Fraumeni syndrome; Classical and Chompret criteria with the Chompret criteria being less restrictive. Individuals meeting Revised Chompret criteria have an estimated ~30% risk of harboring a pathogenic *TP53* variant (Bougeard, et al 2015; PMID: 26014290).

- Members of the *TP53* VCEP calculated likelihood ratios for a patient meeting Classic LFS or Revised Chompret criteria using multigene panel testing from Ambry Genetics Laboratory. Our data showed that individuals meeting Revised Chompret criteria had a LR of >2.08 to ≤4.3 and individuals meeting the Classic LFS criteria had a LR of >4.3 to ≤18.7.



- Therefore, we recommend using the following point system for determining the weight of PS4 evidence:

    - Proband meeting Revised Chompret criteria = 0.5 point
    - Proband meeting classic LFS criteria = 1 points

PS4 Evidence Strength	# of Points Required
PS4	4 or more points
PS4_Moderate	2-3 points
PS4_Supporting	1 point

- If by expert opinion, and justified by consideration of internal laboratory data from large scale multigene panel testing, higher point levels may be awarded (2 instead of 1 for a patient meeting classic LFS, 1 instead of 0.5 for a patient meeting Revised Chompret).

- Please note: This rule code cannot be applied when a variant also meets BA1 or BS1 criteria.

MODERATE EVIDENCE OF PATHOGENICITY

PM1 Located in a mutational hot spot

- There are several known hot spots in the *TP53* gene. This code can be used for variants within the following codons: 175, 245, 248, 249, 273, 282. Use transcript NM_000546.4.

- This code can also be used for germline variants seen in cancerhotspots.org (v2) with ≥ 10 somatic occurrences for the same amino acid. This follows the recommendation from the ClinGen Germline/Somatic Variant Curation Subcommittee (PMID: 30311369).

PM2_Supporting Absent from population databases

- The variant must be absent from population databases. gnomAD is the preferred population database at this time (http://gnomad.broadinstitute.org). The most recent version of gnomAD with a non-cancer subpopulation should be used; however, other versions may be utilized if there is reason to believe they would provide necessary information for curating the variant.

PM3 Variant detected *in trans* with a pathogenic variant

- This rule does not apply to TP53/Li_Fraumeni syndrome

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

- This rule should not be used at this time due to limited data.

PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

This code can be applied for a missense change at an amino acid residue where one or more pathogenic missense changes have been identified. Likely pathogenic missense changes do not apply. The other variant(s) must be asserted as pathogenic by the ClinGen *TP53* VCEP. Grantham should be used to compare the variants. The variant being evaluated must be equal or worse than at least one of the known variants. Splicing should be ruled out. This rule cannot be used in combination with PM1.

PM5: This evidence code can be applied when there are ≥2 pathogenic variants at the same residue.
PM5_Supporting: This evidence code can be applied when there is a pathogenic variant at the same residue.

PM6 Assumed *de novo*, but without confirmation of paternity and maternity (see PS2/PM6 above).

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease (evidence code dependent on number of meiosis and families reported)

PP1: Cosegregation must be observed in 3-4 meioses in 1 family to apply this rule.
PP1_Moderate: Cosegregation must be observed in 5-6 meioses in 1 family to apply this rule.
PP1_Strong: Cosegregation must be observed ≥7 meioses across >1 family in to apply this rule.

PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

- This rule does not apply due to the high number of benign missense variation.

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product

- PolyPhen2 and SIFT *in silico* modeling programs should not be used for this gene.
- Concordance of two predictors is recommended for this gene:
    - Missense variants: according to a published study by Fortuno et al., 2018 comparing the performance of different bioinformatics tools for *TP53* (PMID: 29775997), the tools selected are aGVGD and BayesDel. Please refer to the cited manuscript for further details.
    - Splicing variants: Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc).;)

PP3_Moderate: aGVGD Zebrafish Class C65 required and BayesDel score ≥ 0.16
PP3: aGVGD Zebrafish; Class C25 and higher and BayesDel score ≥ 0.16

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

- Use PS4 to allow for proband counting instead of PP4.

PP5 Reputable source reports as pathogenic

- Do not use this rule code

STAND-ALONE EVIDENCE OF BENIGN IMPACT

Related publication(s): PMID: 33300245 **Date Approved:** August 6, 2019

BA1 Allele frequency is greater than expected for disorder

- Use a minor allele frequency cutoff of ≥0.001 or 0.1% (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

- To set the stand-alone benign MAF cutoff, we used the MAF cutoff established for BS1 (see below) and increased 0.0003 one order of magnitude to come to a value of 0.001.

STRONG EVIDENCE OF BENIGN IMPACT

BS1 Allele frequency is greater than expected for disorder

- Use a minor allele frequency cutoff of ≥0.0003 but <0.001 (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

- To set the strong benign MAF cutoff, we used a prevalence of 1 in 5,000 from Lalloo, et a 2006 (PMID:16644204). We set the genetic and allelic heterogeneity at 100% and penetrance at 30%.

BS2 Observed in heterozygous state in healthy individuals

Using *TP53* multigene panel testing results from two diagnostic labs, we compared the proportion of cancer-free individuals by age 60 in *TP53* carriers versus *TP53*-negative controls. Based on the correspondence between likelihood ratios of pathogenicity and different levels of strengths for ACMG/AMP rules in the study by Tavtigian et al., 2018 (PMID: 29300386), our most conservative results support the following:

- BS2 – This evidence code can be used when a variant is observed in ≥8 females who have reached at least 60 years of age without cancer. These individuals all must have come from a single source (single lab, database, etc). Cases cannot be counted across sources.

- BS2_Supporting – This evidence code can be used when a variant is observed in 2-7 females who have reached at least 60 years of age without cancer. These individuals all must have come from a single source (single lab, database, etc). Cases cannot be counted across sources.

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing

- BS3: Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a functional allele (76-140% activity) or supertransactivation function **AND:**

- There is no evidence of dominant negative effect (DNE) + no evidence of LOF from Giacomelli, et al data

-OR-

- There is a 2nd assay showing retained function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays)

- BS3_Supporting: Transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) that demonstrate a partially functioning allele (>20-and <=75% activity) **AND:**

- There is no evidence of DNE + no evidence of LOF from Giacomelli, et al data.

-OR-

- There is a 2nd assay showing retained function

Do not use code with conflicting evidence

- BS3_Supporting: When there are no transactivation assays (IARC classification based on data Kato et al, 2003) available **BUT:**

- There is no evidence of DNE + no evidence of LOF from Giacomelli, et al data.

-AND-

- There is a 2nd assay showing retained function

Do not use code with conflicting evidence

BS4 Lack of segregation in affected members of a family

- This evidence code can be used in either scenario below:

- The variant segregates to opposite side of the family who meets LFS criteria, or
- The variant is present in ≥3 living unaffected individuals (at least 2 of which should be female) above 55 years of age.

SUPPORTING EVIDENCE OF BENIGN IMPACT

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease

- This rule code does not apply to these genes, as truncating variants account for only a portion of disease causing variants.

BP2 Observed *in trans* or *in cis* with a pathogenic variant

- This evidence code can be applied in either scenario below:

  - Variant is observed *in trans* with a pathogenic or likely pathogenic *TP53* variant (phase confirmed), or
  - When there are 3 or more observations with a pathogenic or likely pathogenic variant when phase is unknown. In this scenario, the variant must be seen with at least two different pathogenic/likely pathogenic *TP53* variants.

BP3 In-frame deletions/insertions in a repetitive region without a known function

- Do not use this rule at this time.

BP4 Multiple lines of computational evidence suggest no impact on gene/gene product

- PolyPhen2 and SIFT *in silico* modeling programs should not be used for this gene.
- Concordance of two predictors is recommended for this gene:

  - Missense variants: : according to a published study by Fortuno et al., 2018 comparing the performance of different bioinformatics tools for *TP53* (PMID: 29775997), the tools selected are aGVGD (Zebrafish; Class C0 or C15 is considered evidence of non-pathogenicity) and BayesDel <0.16 is considered evidence on non-pathogenicity
  - Splicing variants: Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc).

BP5 Variant found in a case with an alternate molecular basis for disease

- This rule code is not recommended for use at this time.

BP6 Reputable source reports as benign

- Do not use this rule code

BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

- Evidence of splice effect on a splice metapredictor (SpliceAI, VarSEAK, etc) is required to use this evidence code. If a new alternate site is predicted, compare strength to native site in interpretation.